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# Replicative adenoviruses for cancer therapy

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**Rapid advances are being made in the engineering of replication-competent viruses to treat cancer. Adenovirus is a mildly pathogenic human virus that propagates prolifically in epithelial cells, the origin of most human cancers. While virologists have revealed many details about its molecular interactions with the cell, applied scientists have developed powerful technologies to genetically modify or regulate every viral protein. In tandem, the limited success of nonreplicative adenoviral vectors in cancer gene therapy has brought the old concept of adenovirus oncolysis back into the spotlight. Major efforts have been directed toward achieving selective replication by the deletion of viral functions dispensable in tumor cells or by the regulation of viral genes with tumor-specific promoters. However, the predicted replication selectivity has not been realized because of incomplete knowledge of the complex virus–cell interactions and the leakiness of cellular promoters in the viral genome. Capsid modifications are being developed to achieve tumor targeting and enhance infectivity. Cellular and viral functions that confer greater oncolytic potency are also being elucidated. Ultimately, the interplay of the virus with the immune system will likely dictate the success of this approach as a cancer therapy.**

Keywords: replicative adenovirus, virotherapy, oncolysis



The concept of virotherapy, an approach to the treatment of cancer with viruses, was inspired early in this century by the observation of occasional tumor regressions in cancer patients suffering from virus infections or receiving vaccinations[1]. Many different viruses were subsequently tested in clinical trials as lytic agents of tumor cells, but a low efficacy/toxicity ratio led to their abandonment. Soon after its isolation in 1953, adenovirus was also tested as an oncolytic agent because of its prolific growth in human epithelial cell lines. In this early trial, cervical carcinomas of 30 patients were injected with different adenovirus serotypes. The results paralleled those obtained with other viruses: an initial tumor regression followed by tumor progression, with a response inversely correlated with the antiviral immune response[2]. The lack of evident therapeutic efficacy also led investigators to dismiss adenovirus as an antitumoral agent.

Despite this initial disappointment, several viruses have recently come forth again as promising anticancer agents. The increasing knowledge about virus–cell interactions has shed light on the natural tropism of some viruses toward tumor cells. For instance, reovirus requires an activated *ras* pathway for infection[3], whereas the autonomous parvovirus life cycle is limited to actively replicating cells[4]. Likewise, several natural and engineered mutants of the herpes simplex virus type 1 can replicate only in dividing cells[5]. Adenovirus has also emerged as a virus that can be engineered with oncotropic properties, as a result of increasing knowledge of adenoviral interactions with cell cycle regulatory proteins and the experience gained from its use as a gene delivery vehicle. Even though the adenovirus tumor selectivity can be tackled at different levels, perhaps the area that has concentrated most research efforts so far is the development of conditionally replicative adenoviruses (CRAds) designed to replicate exclusively in tumor cells. Improving the delivery of CRAds, both to local-regional and disseminated disease, as well as the virus intratumoral spread are growing research areas. Last, but not least, the study of the interaction of replicative adenoviruses with the immune system is mandatory in order to improve the outcome of viral oncolysis. Here, we review the studies performed on replicative adenoviruses at these different levels and provide some insights for future studies (see Fig. 1).

## Adenovirus tumor-selective replication

Since the leading efforts of Onyx Pharmaceuticals (Richmond, CA), the study and design of adenoviruses that replicate selectively in tumor cells is the area of most intensive research in adenovirus-based cancer virotherapy[6]. Before analyzing what has been achieved in selective replication, it is useful to comment on the assays that have been employed.

The selective replication of a CRAd has been generally studied by comparing different human cell lines in vitro. Whereas different cell lines may show various levels of susceptibility to adenovirus infection and virus production, these variables have not always been considered. This deficiency can be corrected by including a comparison with a nonselective adenovirus like the wild type (Adwt) (see below), albeit not all studies have incorporated this control. The effect of a CRAd in vitro has been measured at the level of viral DNA replication, late gene expression, cell death, and progeny production. These parameters are not always correlated. Viral DNA replication, hexon expression, and cell death can occur without progeny production. Conversely, in some cell types, such as fibroblasts and keratinocytes, virus production can occur without evident cytopathic effect. Therefore, although crystal violet staining, trypan blue exclusion, and other live-dead assays yield information about the CRAd effects in each cell type, from an oncolysis standpoint progeny production is most relevant. Furthermore, the progeny released to the supernatant is likely to have more oncolytic predictive value than if we measure it after releasing the virus from the cells by freeze/thaw cycles. For a given cell type, the burst size or number of infectious particles produced per cell is a quantitative measure of progeny production.

Perhaps the best and simplest way to numerically express the selectivity of a CRAd between a tumoral and a normal cell line, could be the following:

$$\left(\frac{\text{CRAd burst size in tumor cell}}{\text{CRAd burst size in normal cell}}\right) \times \left(\frac{\text{Adwt burst size in normal cell}}{\text{Adwt burst size in tumor cell}}\right)$$

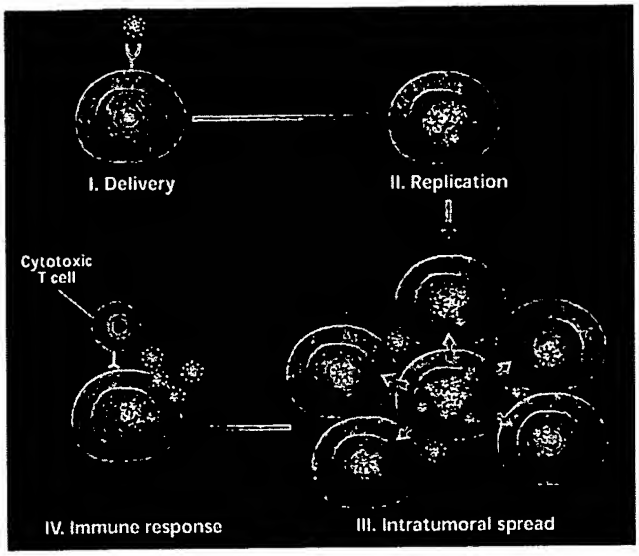


**Figure 1. Oncolysis steps that provide opportunities for intervention.**

Using this formula, if a CRAd produces 1,000 plaque-forming units (p.f.u.)/cell in a tumor cell and 1 p.f.u./cell in a normal cell, and Adwt produces 10,000 and 100, respectively, then this CRAd is 10-fold more selective than Adwt. The presence of Adwt in this formula corrects for difference in infectivity and virus production between the different cell types. On the other hand, the choice of normal cells is critical. Primary human embryo kidney cells are the most sensitive to adenovirus types 2 and 5 infections[7]. Human fibroblast cell lines and endothelial cell lines, employed in different studies[8–10], show poor infectivity and delayed cytopathic effect. Mammary (MEC) and bronchial (NHBE) epithelial cells used in other reports[9,11] are more permissive to adenovirus infection and production. In fact, the effect of the CRAd on normal cells, particularly those of epithelial origin that could become infected when targeting a particular tumor, should be analyzed although their availability has so far limited such studies.

Many levels in adenovirus replication may be regulated for the purpose of generating a CRAd. In recent years, two major strategies have come forth. In the first one, viral genes that become dispensable in tumor cells, such as the genes responsible for activating the cell cycle through p53 or Rb binding, have been completely or partially deleted. In the second strategy, transcription of viral genes has been controlled by replacing the native viral promoters with tumor-specific promoters (tsp) (see Table 1 for the different types of CRAds developed so far). Mutants defective at other levels such as intracellular trafficking, nuclear import of the viral genome, RNA splicing, nuclear export of RNA, or protein translation are conceptually CRAd candidates. For example, a virus in which the splicing of a viral gene or an interfering stop signal is regulated like the tumor-associated splice variant of CD44 could be tumor selective[12]. Nonetheless, we have learnt that, in reality, achieving tumor-selective replication is not so simple. McCormick's group at Onyx proposed that an E1b-55kDa-deleted adenovirus would replicate selectively in *p53*-deficient cells, an alteration common in tumors. The protein encoded by the *E1B 55K* gene binds and inactivates *p53* in normal cells in order to initiate virus replication. Therefore, only cells that have lost *p53* are permissive for CRAd replication, because there is no requirement for the viral E1b-55kDa protein in switching off *p53*[13]. When a few cell lines were compared, this was found to be the case.

In *p53*$^-$ cell lines C33A and U373, the mutated virus dl1520 (Onyx-015) was shown to lyse cells at similar levels as the wild-type virus Ad5wt, but was 100-fold less effective in lysing the *p53*$^+$ cell line U87. The same result was observed for the matched pairs of cell lines U2OS55K/U2OS and RKOp53/RKO[13]. However, lytic assays from the same group revealed exceptions to the lack of replication of dl1520 in *p53*$^+$ cell lines, such as HepG2, HlaC, and HCT116 (ref. 9). When titering the viral progeny, Rothmann and colleagues[11] have also reported a lack of correlation between p53 status and dl1520 replication[11]. Contrary to the conclusions from lytic assays, U373 (*p53*$^-$) produced 100-fold lower levels of dl1520 than wild-type adenovirus, and U87 (*p53*$^+$) produced as much dl1520 as the wild type. In this report, the amount of transcriptionally active p53 was determined using a p53-responsive reporter plasmid, to rule out the possibility that *p53*$^+$ cell lines could have p53 inactivated through mechanisms such as *MDM2* overexpression. Furthermore, in *p53*$^-$ cells as well as normal primary cells, the differences in progeny production were reduced when infecting with more infectious particles per cell, confirming the fact that E1b becomes dispensable at high multiplicities of infection.

Hay and colleagues[8] have studied viral DNA replication, viral protein synthesis, host cell protein shutoff, cytopathic effect, and progeny production of the dl338 E1b-55kDa mutant, and found no correlation with p53 status. Besides a lack of correlation between dl1520 progeny production and p53 status, Goodrum and Ornelles[14] have also described that S-phase cells are more susceptible than G1-phase cells to cell killing by dl1520, but not wild-type adenovirus[14]. These observations seem to be related to other functions of the E1b-55KDa protein. The initial studies of this viral protein indicated that *E1B 55K* mutants are impaired in p53-defective cells[15,16]. The defect is attributed to the reduced rate of late viral protein synthesis due to impaired nuclear RNA export mediated by the E1b-55kDa–E4-34kDa complex. A defect in viral mRNA translation can also account for the p53-independent impairment of dl1520[17].

It is conceivable that cells in S phase are less dependent on this complex to export or translate viral RNA, and thus dl1520 has certain specificity for dividing cells. Mutants affected in *p53* binding, but not in other E1b-55KD functions, may result in the desired conditional progeny production upon p53 absence. However, these functions are not easily separable in the protein sequence. Another step toward restricting replication to p53-deficient cells may be the deletion of the E4-34kDa protein domains that also inactivate p53. On the other hand, the role of p53 in adenovirus propagation is a question that remains to be answered. If p53 is necessary for efficient release of progeny from the infected cell[18], other genes involved in cell lysis that can complement the p53 defect will need to be incorporated into CRAds designed for *p53*$^-$ tumors.

Currently, dl1520 (ONYX-015) has reached phase I and II clinical trials for head and neck, pancreatic, ovarian, colorectal, lung, and oral carcinomas. In these trials, up to $2 \times 10^{13}$ viral particles have been administered systemically or locally, but have brought about no objective tumor responses[6]. Efficacy rather than toxicity seems to be the limitation. Combination with chemotherapy or the insertion of therapeutic genes in the virus have increased the efficacy in animal models[19,20]. Incorporation of the thymidine kinase gene in a CRAd has been shown to confer therapeutic efficacy and safety traits. However, the effects of radiation, chemotherapy, or cytotoxic gene therapy on viral replication and the timing of these auxiliary interventions with respect to the oncolytic treatment have not been evaluated yet, and it would be also desirable to understand the limitations of the single agent before trying complex combinations.

A mutant also proposed for specific replication is based on the deletion of the retinoblastoma gene (*Rb*)-binding site of E1a[21]. These mutants cannot induce resting cells to pass the G2/M checkpoint and progress to mitosis[22]. One of these mutants AdΔ24 has been studied for oncolysis of glioblastomas. Cells arrested by the previous infection with a pRb-expressing adenoviral vector become refractory to cell lysis by AdΔ24. In a brain tumor context where normal cells are resting, the specificity of this agent could be enough to allow a certain level of amplification in the dividing tumor cells. Mutants unable to bind Rb and p300 will likely be even more tumor-selective because of their inability to induce S phase. The lack of good animal

Table 1. Types of adenoviruses used as oncolytic agents

| Name (serotype) | Basis of tumor-selective propagation | Therapeutic traits | Reference |
|---|---|---|---|
| Ad wild type (various serotypes) | None | Oncolysis | 2, 51 |
| Ad5/IFN (Ad5) | None | Oncolysis & immunostimulatory gene therapy | 54 |
| dl1520 or Onyx015 (Ad2/5) | E1b55kDa-deletion abrogates *p53* binding | Oncolysis | 13 |
| AdTK$^{RC}$ | E1b55kDa-deletion abrogates *p53* binding | Oncolysis & suicide gene therapy (TK) | 19 |
| Ad-5-CD-TKrep or FGR (Ad5) | E1b55kDa-deletion abrogates *p53* binding | Oncolysis & suicide gene therapy (CD + TK) | 55 |
| AdvE1AdB-F/K20 (Ad5) | E1b55kDa-deletion abrogates *p53* binding | Oncolysis with enhanced infectivity | 41 |
| AxE1AdB (Ad5) & AdCAhIL-2 (Ad5) | E1b55kDa-deletion abrogates *p53* binding | Oncolysis & immunostimulatory gene therapy | 31 |
| AdD24 (Ad5) | E1a deletion abrogates *Rb* binding | Oncolysis | 21 |
| CN706 (Ad5) | Regulation of E1a under the PSA promoter | Oncolysis | 24 |
| CN763 (Ad5) | Regulation of E1a under the kallikrein 2 promoter | Oncolysis | 10 |
| CN764 (Ad5) | Regulation of E1a under the PSA promoter and E1b under the kallikrein 2 promoter | Oncolysis | 10 |
| CV739 | Regulation of E1a under rat probasin promoter and E1b under human PSA promoter | Oncolysis | 29 |
| CV787 | Regulation of E1a under rat probasin promoter and E1b under human PSA promoter | Oncolysis (enhanced compared with CV739 due to the presence of E3) | 29 |
| AvE1a041 | Regulation of E1a under the AFP promoter | Oncolysis | 23 |
| GT5610 (Ad5) + AdHB (Ad5) | Regulation of E1a under the AFP promoter | Oncolysis | 30 |
| DI337 (Ad5) | None | Oncolysis (enhanced due to E1b-19kDa deletion) | 43 |
| DI316 (Ad5) | The complete deletion of E1a makes this mutant dependent on intrinsic or IL-6-induced E1a-like activity | Oncolysis | 56 |
| DI118 (Ad5) | The complete deletion of E1b abrogates p53 binding; however, E1a-induced apoptosis is not inhibited by E1b-19kDa | Oncolysis | 44 |

models that allow adenovirus replication in normal cells is an obstacle to evaluate the toxicity of this and other CRAds.

A second strategy to achieve tumor-selective replication is the replacement of viral promoters with tumor or tissue-specific promoters. Paul Hallenbeck (Genetic Therapy–Novartis; Gaithersburg, MD) and Daniel R. Henderson (Calydon; Sunnyvale, CA) have pioneered the efforts in this direction using α-fetoprotein (AFP) and prostate-specific antigen (PSA) promoters to drive the adenovirus *E1a* gene[10,23,24], to treat hepatocellular and prostate carcinomas, respectively. Even though they have shown some degree of specificity with these promoters, it is known that cellular promoters do not keep the proper fidelity in the viral genome[25]. Low levels of viral products such as E1a may be sufficient for replication, thus preventing specificity. Different mechanisms can account for this leakiness: the presence of enhancers in the viral genome, the presence of viral DNA-binding protein instead of histones, and the location of the viral genome in active centers of transcription, among others. Insulation of promoters with genomic DNA sequences has been shown to increase the promoter fidelity[26].

In a different strategy, the interference of *cis* sequences has been avoided by delivering the regulated viral gene expression cassette in a plasmid, allowing only one round of viral replication[27]. The regulation of viral genes for which the products are needed in greater amounts, or of several viral genes that participate in one viral function (e.g., the role of E1b-55kDa and E4-34kDa in RNA transport), could further limit viral replication. In this regard, a double-regulated E1a/E1b adenovirus by two different prostate-specific promoters has been reported and shown greater attenuation in non-prostate tumor cells[10]. If promoter fidelity in the viral context can be improved, promoter regulation is attractive because it does not rely so much on our knowledge of viral functions. In addition, this strategy has the advantage, compared with E1a mutants, that the absence of E1a expression in normal cells may decrease E1a-associated toxicity.

In the strategies mentioned above, the size of the exogenous DNA that a CRAd can accommodate is limited. Whereas early region 3 is dispensable for virus replication, it is actually necessary for proper cell lysis[28] and to partially evade the immune system[29]. Therefore, a CRAd with no deletions can only accommodate an extra 1.8 kb of DNA. To avoid this limitation, a double system composed of both a gutless 36-kb capacity adenovirus containing an insulated AFP-E1 cassette and an E1-deleted adenovirus has been used and shown to selectively lyse AFP-expressing tumors[30]. In a similar complementation strategy, a CRAd such as Onyx-015 has been coinjected with an E1-deleted vector expressing interleukin-2 to increase intratumoral expression and therapeutic outcome[31]. However, the requirement of double infection curtails the efficacy at low multiplicities of infection as would be the case after systemic delivery, or in poorly infectable tumors.

## Delivery and intratumoral spread

Although nonreplicative adenoviral vectors have been the subject of numerous modifications for efficient and selective delivery to tumors, little has been done on the delivery of replicative adenovirus. Nonetheless, the rules that apply to the delivery of adenoviral vectors can also be applied to CRAd delivery. A proper delivery should spare normal cells from infection to avoid toxicity, so it includes the concept of tumor targeting. The delivery requirements to local or disseminated cancer are, however, very different. For localized malignant disease, tumor targeting of replicative adenoviruses may not be critical. Local-regional ovarian carcinomas, head and neck, and brain tumors can be approached from a direct injection route with chances of success. For disseminated cancer, however, systemic delivery is necessary.

Adenovirus is not a blood-borne virus and its clearance from blood is very effective[32]. Like many other viruses, particles, and colloids, adenovirus is rapidly cleared by liver Kupffer cells by an unknown mechanism that seems independent of the interaction of its capsid fiber with the Coxsackie-adenovirus receptor (CAR). Other CAR-dependent cellular interactions, such as those with hepatocytes, could be bypassed by using CAR-binding ablated adenoviruses[33]. In this way, CAR-binding ablation could lessen the hepatocellular toxicity associated with adenovirus systemic delivery. Furthermore, other capsid modifications based on the alteration of hydrophobicity or charge are conceivable in order to increase virus persistence in blood, which in turn would facilitate virus delivery to tumors.

The porosity of the endothelial barrier could account for certain levels of passive tumor targeting, since, with the exception of spleen

and liver vessels, only tumor vessels may allow the extravasation of particles in the adenovirus size range. This is the principle behind the systemic delivery of drugs by long-circulating stealth liposomes[34]. On the other hand, active tumor targeting of adenovirus has been achieved using antibodies or other ligands, such as epidermal growth factor and basic fibroblast growth factor, attached to its capsid[35,36]. In vivo stability and size of these complexes are caveats that need to be addressed. A better strategy is to genetically modify the fiber or another exposed capsid protein to present a tumor-specific ligand. Progress in this direction has been encouraged by the successful insertion of small peptides such as the Arg-Gly-Asp (RGD) motif in the adenovirus fiber[37], resulting in adenovirus retargeting.

Finally, nonviral delivery of the replicative agent genome can be considered as another way to circumvent the blood clearance, targeting, and toxicity limitations associated with adenovirus particles. In this regard, linear adenovirus DNA is poorly infective, especially when the terminal protein attached to the ends that act as a replication primer is not preserved after DNA purification. Circular adenovirus genomes fused at their termini form infectious plasmids. If the infectivity of these plasmids could be increased by modifying the terminal repeats so they can efficiently dock the replication initiation complex, then these plasmids could be delivered by means of nonviral vectors optimized for systemic administration and tumor targeting.

Intratumoral spread is a problem different from delivery. As mentioned above, selective tumor delivery requires the ablation of the natural tropism of adenovirus, determined by its binding to CAR[33]. In contrast, to enhance intratumoral spread, it is desirable to broaden the tropism to different entry pathways, avoiding the selection of noninfectable tumor cells. Another difference is that, whereas delivery can be accomplished by conjugation of the virus to ligands, intratumoral spread will require the capsid modifications to be genetically incorporated in order to be present in the progeny. The function of the natural adenovirus type 5 receptor is not known, but its expression seems ubiquitous. However, some reports have indicated that in certain cases tumors may have low CAR levels[38,39]. To broaden the viral tropism, ligands have been incorporated into the fiber without interfering with CAR binding. The HI loop and the C terminus of the adenoviral fiber allow these insertions. The insertion of small peptides, such as stretches of lysines to bind heparan sulfate and polyanionic cellular receptors, as well as peptides containing the RGD motif to bind αv integrins have been shown to broaden viral tropism and increase infectivity[37,40]. In a CRAd context, the insertion of a polylysine at the fiber C terminus increased the oncolytic potency of Onyx-015 in vitro and in vivo by intratumoral administration in the glioblastoma cell line U373MG[41]. However, as described for several viruses, strains or mutants selected for enhanced or widespread binding in vitro may not target specific cell types after systemic administration[42].

Another approach to increase the viral spread has been to enhance the release of the replicative vector from the tumor cells. A replicative adenovirus deleted in the anti-apoptotic E1b-19kDa viral gene induces more apoptosis, is released earlier, and spreads faster than wild type[43]. The combined deletions of E1b-55kDa and E1b-19kDa could therefore enhance the oncolytic effect of E1b-55kDa mutants, such as Onyx015. An adenovirus containing this complete E1b deletion has been shown to be cytotoxic[44]. The effect of the E1b-19kDa deletion on progeny production is, however, variable among different cell lines, and the effect of premature apoptosis on progeny production remains an issue. Moreover, the possible induction of apoptosis in normal cells with the subsequent associated toxicity should be taken into account. Rather than deleting viral inhibitors of cell death, such as E1b-19kDa, one could seek to enhance the mechanism by which adenovirus promotes cell death. Preserving the adenovirus E3-11.6kDa death protein[28] in a CRAd has been shown to enhance its particle release and oncolytic potency[29]. Proteolysis of keratins by L3-23kDa and inhibition of cell translation by L4-100kDa also promote cell lysis and progeny release[45]. The dependence of the oncolytic potency of CRAds on these viral functions remains to be studied.

A different area of intervention to be explored is the diffusion of CRAds throughout the tumor. Several vasoactive drugs, cytokines, or physical treatments, such as radiation or heat, increase tumor vascular permeability and blood flow, leading to faster diffusion[46]. The incorporation of lytic enzymes, such as hyaluronidase, into a CRAd could also increase diffusion rates.

## Control of the immune response

As mentioned earlier, the major obstacle for a successful virotherapy is the neutralizing immune response. In the early clinical trials with wild-type adenovirus, less frequent therapeutic responses were observed in patients with elevated serum neutralizing antibodies[2]. Cortisone was administered to lessen the immune reaction and although it seemed to enhance the initial intratumoral necrosis, it did not affect the antibody production. In Onyx-015 trials, where a well-purified virus has been used, it seems that at least pre-existing antibody levels have not influenced antitumoral activity or toxicity[6]. Although oncolytic/immune response kinetics need to be addressed meticulously in clinical trials to understand CRAd limitations, an area that requires improvement is the development of an appropriate preclinical model.

Preclinical studies on the interaction of the immune system with replicative adenoviruses have been limited by the lack of appropriate animal models, because of the poor replication of human adenovirus in other species, including monkeys. For this reason, only immunodeficient murine models have been employed to study oncolytic potency in xenografted human tumors. Perhaps the only preclinical studies on the effect of the immune system in virotherapy come from models of oncolysis by herpesvirus hrR3 in rodents, where the virus can replicate. In one report, innate preimmune IgM and neutralizing antibodies have precluded successful oncolysis of brain tumors in rats[47]. In contrast, no differences have been found treating liver metastases with hrR3 in immunocompetent or immunodeficient mice[5].

Ad5 has been administered oronasally into different species to reproduce the pathology of human infections. Cotton rats and pigs have been identified as the most permissive models for Ad5 replication, which occurs to some extent in lungs[48,49]. Although some conclusions could be obtained regarding the efficacy of oncolytic adenoviruses in these immune-competent hosts, these models are not permissive enough to test the toxicity of adenovirus, as indicated by the positive correlation between titers in tissues and input dose of Ad5. In truly permissive species, viral titers in tissues are independent of the dose injected. In general, murine and simian cells allow Ad5 DNA replication, but a late block prevents progeny production[7]. However, there is some evidence that mice support a low level of replication of Ad5 (ref. 50). After a high intravenous dose in mice and hamsters, Ad5 undergoes an abortive, but lytic, infection in many hepatocytes, whereas only a few are able to complete virus production at low levels[32]. Khoobyarian and colleagues[51] observed Ad2 progeny production in hamster melanoma cells and applied this model to oncolysis. Only high doses of Ad2 injected soon after the implantation of the tumors were effective. Poor permissiveness of hamster cells could account for this lack of efficacy.

In an attempt to find a good murine model for CRAds, Ganly and coworkers[52] recently have studied the replication of Ad2 in murine cells. Two cell lines, B9 and SN161, were shown to produce progeny at levels 50-fold and 25-fold (respectively) lower than the human A2780Cp cell line, but no comparison to the commonly used human 293 cell line was provided. This work may lead to immune-competent animal models where some virotherapy could be achieved, opening the possibility to study and modulate the immune system. It is conceivable that neutralizing antibodies will counteract the spread of the virus and antiviral cytotoxic T lymphocytes will destroy infected cells. In this case, shifting the immune response toward the

T-helper 1 type could change the oncolytic outcome. Interleukin-12 can induce this shift and has been shown to abrogate the development of neutralizing antibodies against adenoviral vectors[53].

## Conclusions

Replicative adenoviruses are being engineered to achieve selective targeting and amplification for the treatment of local and disseminated cancer. Whereas chemotherapy and immunotherapy remain the current therapeutic choices for disseminated tumors, an agent that can be delivered systemically, can be targeted to tumor cells, and can amplify their cytolytic effect in a tumor-specific manner would undoubtedly be of clinical benefit. These agents share some attributes of the immune system with the advantage that tumors have not been exposed to them before their administration and thus have not selected specific mechanisms to evade them. Blood persistence, tumor targeting, tumor-specific replication, lateral spread, and the interaction with the immune system are obstacles that have been identified. Research is ongoing in each of these areas to improve the efficacy/toxicity ratio. Claims of selective magic bullets need to be modest, though, because much remains to be known about the regulation of viral replication and how to harness it.

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